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A STUDY OF THE PHYSIOLOGICAL EFFECTS OF STRONTIUM

Dissertation

Submitted to the School of Hygiene and Public Health of the Johns Hopkins University in Conformity with the Requirements for the Degree of Doctor of Science in Hygiene

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A STUDY OF THE PHYSIOLOGICAL EFFECTS OF STRONTIUM

Strontium belongs to the group of alkaline earths and, therefore, is closely related chemically to calcium and magnesium. It is frequently assumed that within the body strontium exerts effects similar to those of the other two related elements.

The first experimental data which could be found on the substitution of calcium by strontium and magnesium were those of Papillion in the year 1870 (1). Papillion fed one pigeon and two rats rice and gluten, and gave in addition a solution of calcium chloride, potassium nitrate, potassium carbonate, potassium sulphate, and the phosphates of the above earths. Just which salts of strontium and magnesium, and the amounts of these, he fed are not given. He obtained the following results, and his conclusions were that the calcium of the bone could be replaced by strontium or magnesium or other alkaline earth or earth metals.

	Pigeon		Rat I		Rat II	
Food	Strontium		Magnesium		Aluminum Phosphate	
Duration of feeding	7 months		70 days		74 days	
In bone ash	CaO 46.75%	SrO 8.45%	CaO 46.15%	MgO 3.56%	CaO 41.10%	AlO 6.95%

In 1872 H. Weiske (2) fed rabbits one hundred days with hay and turnips. His experiments were made on eleven groups of animals. One was a control group which received no added mineral salts. Two groups each, one of grown animals and one of young animals, received, respectively, calcium phosphate, magnesium phosphate, and strontium phosphate.

Weiske was not able to find one trace of strontium in the ash of these bones. He could find no appreciable change in the ash content of his animals over that of the control group. The results of his normal and magnesium fed animals may serve to illustrate all.

	Calcium oxide	Magnesium
1. Bone ash of normal rabbits	53.94-53.77	1.06-1.23
2. Bone ash of rabbits receiving one gm. daily of magnesium phosphate	54.21-53.68	1.09-1.24

In 1874 J. König questioned this work (3). He thought that Weiske probably got no change in the bone ash when supplementary salts were added because the food contained a sufficient amount of normal bone-building elements, and any excess of these or any others were simply discarded by the animal body. Modern investigations do not support the view of König that the animal body can effectively rid itself of excessive amounts of mineral elements without unfavorable effects. He repeated Weiske's work, giving each of his animals one hundred grams per day of a mixture of wheat gluten, starch, sawdust, and carrots. This diet is very low in its mineral content. His results are interesting.

Diet	Duration	Percentage Content in Bone Ash			
		CaO.	SrO.	MgO.	H3PO4
Young rabbits (5 weeks old) normal diet.		52.73%	----	1.62	40.92
No. 1. Calcium phosphate	70 days	51.36	----	0.70	42.54
No. 2. " "	73 "	51.92	----	0.82	42.50
No. 3. Strontium phosphate	23 "	44.77?	5.21	0.64	39.64
No. 4. " "	27 "	49.27	4.71	----	40.68
No. 5. " "	20 "	46.78	5.37	1.09	39.47
No. 6. Magnesium phosphate	31 "	51.60	----	1.48	39.51
No. 7. " "	82 "	51.92	----	1.68	42.27

This criticism of König's led Weiske to repeat and extend his work on strontium (4). In his new experiment he fed four groups of rabbits from the 13th of October until the 10th of December. One group received calcium-free barley, the second the calcium-free barley plus strontium phosphate, the third the calcium-free barley plus magnesium phosphate, and the fourth received normal food. It is interesting to note that the loss in body weight of groups one, two, and three were practically identical, and that the percentage loss in weight of two animals which he starved to death was no greater than

that of some of the animals receiving the no calcium, the strontium, and the magnesium diets.

Weiske took pains to free the bones which he used for chemical analysis from fat and marrow elements, but again he was unable to demonstrate any strontium in the bone ash, and the magnesium content of the ash of the animals receiving the magnesium phosphate diet had no increase of magnesium over the normal. He criticises König's results on two grounds. First, he says that all the strontium which König found might possibly have been in the fat and marrow elements of the bone, and secondly, that the analytical method used by König was probably faulty.

Both criticisms are weak in that Weiske does not prove that strontium was present in the marrow elements, and the differences which he describes between his own method of analysis and König's are insignificant.

The method which Konig used is in vogue to-day as a government method, and its sources of error will be discussed later under 'Chemical Methods".

Previous to this, Weiske's experiments concerning the role of calcium and phosphoric acid in bone formation led him to question if the content of these two elements in the diet could not be varied in such a manner as to produce rickets and osteomalacia. In 1873 Weiske and Wildt (5) arrived at the far reaching conclusion that rickets was caused by a lack of certain mineral elements, particularly calcium.

These experiments of Weiske's excited considerable attention. Other workers experimented with calcium low diets, and in many cases reported that rickets or rickets-like conditions were produced.

Miwa and Stoeltzner (6) raised the question at this point

as to whether rickets could be identified merely by clinical observation or even by the gross anatomical findings. They conducted an experiment which was intended to prove whether the conditions reported by the various observers were due to rickets or some other pathological condition of the bones.

They fed normal growing dogs on horse flesh and fat. The animals were maintained on the diet six months. At the end of that time the legs were bowed, and there were marked epiphyseal swellings and beads at the costochondral junctions. The animals walked with very great difficulty. The authors say "Das Bild entsprach in seiner ausseren Erscheinung durchaus dem bei der Rachitis".

However, the authors pronounced the disease not rickets but a generalized osteoporosis with rickets-like changes in the periosteum and lack of calcification of the proliferating cartilage

They arrived at this conclusion because of the following histological findings. A generalized and excessive amount of osteoporotic tissue, the presence of cartilage and of osteophytes in the periosteum, a slight irregularity of the zone of ossification, a broadening of the band of proliferating cartilage (about double that of normal width), the loss of regular arrangement of the cartilage trabeculae in the growth border, the pushing back of the cartilage matrix by the very abnormally large cartilage cells, and finally, a normal amount of provisional calcification of the cartilage.

In order to differentiate the above picture from that of rickets they summarize the outstanding characteristics of rickets as being, first and foremost, a great excess of osteoid tissue; second, lack, or almost complete lack, of provisional calcification; third, irregularity in the arrangement of the various tissue structures

in the zone of ossification; and last, the proliferation of the periosteum and its impregnation with osteophytes and cartilage, and the broadening of the proliferating cartilage border.

Von Cremer (7) fed animals a low calcium diet and gave in addition strontium phosphate. He says, "das Bild in vielen Punkten dem bei der echten Rachitis ahnlich war". Furthermore, he was unable to detect any difference between his strontium fed animals and his low calcium ones.

In 1909 Lehnert took up the study of the production of experimental rickets by the addition of strontium to a low calcium diet. In discussing the literature he cites an experiment by Korsakov (8) in which Korsakov fed strontium to two pregnant animals, one for twenty-eight days and the other for fifty days prior to delivery. The mothers remained in good condition, but the young showed characteristic symptoms of congenital rickets.

The legs were bowed and the bones fractured and flexible. All the bones were porous and contained much osteoid tissue. The cartilage showed but little of the characteristic rachitic picture.

Korsakov was unable to find any strontium in the bones on chemical analysis.

The lack of detailed information concerning Korsakov's work prevented Lehnert from arriving at any definite conclusions in regard to it. The description of the relatively large amount of osteoid tissue, the pronounced bending of the leg bones, together with the occurrence of disturbances at the proliferating cartilage borders, was one which certainly pointed to a rachitic condition.

Also he was interested in a report by Wegner (9) that when elemental phosphorus was added while feeding a low calcium diet, the picture obtained was somewhat similar to that of human rickets, the chief difference seemingly being in the arrangement

and distribution of the osteoid tissue. Wegner gave to the condition which he found the name of "Phosphorsklerose".

With this data before him Lehnert undertook a careful study of the effect of additions of strontium phosphate (8,9) to the food. Six of his animals were five weeks old puppies. One received low calcium food; the second, low calcium food plus calcium phosphate; the third and fourth, low calcium food plus strontium phosphate; the fifth, customary food plus strontium phosphate; and the sixth, customary food plus strontium phosphate plus calcium phosphate. Also he fed a pregnant dog food low in calcium and strontium phosphate. Three of the young were nourished by the mother for two weeks. In another experiment he fed four pregnant rabbits their customary food with the addition of strontium phosphate. The thirty-two young born were studied histologically. Finally, he fed a grown dog for six months his customary food with large

doses of strontium phosphate.

He found that some of the young of the strontium fed rabbits when born had very bowed legs and showed other external appearances of rickets. The rest appeared to be normal. Some of the young after nursing also showed deformities of the bones. Lehnert concluded that strontium may enter the animal body through both the blood and the milk.

Histological examination showed an increase of osteoid tissue, and some disturbance of the proliferating cartilage border. There was lacking the typical rachitic irregularity of the proliferating cartilage and abnormal vascularization of the epiphyseal cartilage. There were many osteoblasts. Also, provisional calcification was at hand.

In his discussion of his data, Lehnerdt cites a theory proposed by Stoeltzner as to the cause of the differences in conditions

giving rise to osteoporosis and rickets. Stoeltzner says that an osteoporotic condition arises when there is an insufficient amount of calcium to build normal bone. The osteoblasts build into bone as much of the osteoid as is possible with the calcium which they have at their disposal. As the bone grows and greater amounts of calcium are demanded, part of the bone already formed is resorbed and the calcium redistributed. Thus, the bone grows progressively more porous and spongy. In true rickets the osteoid tissue is not able to take up calcium, although calcium is present in sufficient amounts.

The chief resemblance of Lehnerdt's histological sections to the sections of true rickets lay chiefly in the large amount of osteoid tissue present. He believed that this osteoid tissue was calcium absorptive and remained only partly uncalcified because the calcium at hand was not sufficient for the calcification of newly built bone tissue.

The evidence available from Lehnerdt's experiments would seem to indicate that this was true. It is evident, however, from the studies of McCollum, Simmonds, Shipley and Park (10) that a diet containing about 0.3 per cent,or three-fourths the optimum amount of phosphorus, and an excessive amount of calcium (1.12 per cent), leads, because of the unfavorable ratio between calcium and phosphorus, to the development of a wide metaphysis, free from calcium salt deposits. This is a situation in which an excess of calcium prevents the deposition of calcium salts, whereas with the same content of phosphorus in the diet and with a lower content of calcium (an improved calcium:phosphorus ratio) calcium deposition is possible. Lehnerdt was not aware of the importance of the ratios between these elements in its relation to calcium salt deposition in the growing bone.

Lehnerdt's descriptions and conclusions are interesting in

the light of present day experiments with rickets. It is to be observed that the diets on which he obtained his pseudo-rachitic picture were those which were low in calcium and high in phosphorus. His rabbits received oats, bran, beets, potatoes, and green food. All of these foods are low in calcium, the fresh foods are also low in phosphorus, but the oats and bran are fairly high, containing 0.38 and 1.35 per cent, respectively. McCollum, Simmonds, Shipley and Park (11) have determined by experiment that a content of phosphorus in the form of phosphate equal to 0.4 per cent and a content of calcium equal to 0.64 per cent of the food represent approximately the optimal concentration of these elements for the nutrition of the rat. The addition of strontium phosphate in Lehnerdt's experiments markedly increased the phosphate ion. His dogs received horse flesh and fat. The fat probably contained no more than traces of phosphorus, and no calcium. One hundred grams

of lean meat contains about 3 mgs. of calcium and 220 mgs. of phosphorus. So it is seen that in the dogs' rations also the phosphorus was greatly increased over that of the calcium by the addition of strontium phosphate.

Lehnerdt could detect little difference between the animals which received calcium phosphate and the control animals which received normal food. His description of his sections from those animals which received the low calcium - strontium phosphate diets resembles in certain particulars one given by Shipley, Park, McCollum, and Simmonds (13) of sections obtained from rats which had received a low calcium, optimal phosphorus diet deficient in the anti-rachitic factor. The description of the latter is as follows:

When the diets in which the calcium was deficient, the phosphorus at a level not far from the optimal, and the calcium: phosphate ratio low, were fed to rats, a diseased condition of the skeleton developed which also bore marked resemblances to the lesions

found in the rickets of human beings. The gross deformities caused by the diets of this type were as great or greater than those caused by diets low in phosphorus and high in calcium, and corresponded exactly to the deformities found in rachitic children. The thorax was deformed; it was flattened from side to side and marked by deep grooves which followed the costochondral junctions; the angular deformities produced at the junction of the costal cartilages and the shafts projected into its interior; the costochondral junctions were enlarged and greatly distorted; fractures in the shafts of the ribs were especially numerous. The lower ends of radius and ulna were enlarged, as were also the ends of all the long bones of the extremities, and the bones were extremely soft and weak. Between the cartilage and the shaft was a white intermediate zone one to three millimeters deep. Microscopic examination showed that the cartilage was entirely or nearly free from calcium and was invaded in an irregular manner by the vascular elements of the shaft. In consequence, the cartilage extended toward the shaft in irregular prolongations. The cells of the cartilage in proximity to the shaft showed evidences of degeneration and metaplasia. The intermediate zone was composed of cartilage in a more or less degenerated state, osteoid trabeculae, blood vessels surrounded by marrow elements, and a few deposits of calcium for the most part

situated near the periphery and connective tissue. The trabeculae of the shaft were bordered by rather broad zones of osteoid. A loosely arranged fibrous tissue invested many of the trabeculae and in those places in which it filled in the spaces between the trabeculae it gave rise to histologic pictures which closely resembled those furnished by the fibrous marrow in the rickets of human beings.

The pathologic condition induced in the bones by this type of diet did not, however, correspond at all points to that usually found in the human subjects of the disease. The metaphysis was composed in larger part of osteoid trabeculae. Though these osteoid trabeculae were free from calcium deposition, they nevertheless retained a certain semblance of orderly arrangement. The osteoid zones about the trabeculae were not always so broad as in the rats on the diets of the high calcium, low phosphorus type, though they were quite as broad as the osteoid borders in the bones of rachitic children. Resorptive activity was exceedingly marked.

The abnormal condition of the skeleton produced by this type of faulty diet corresponded to that found in human beings with rickets in all fundamental respects, namely, the degenerative and metaplastic changes of the cartilage, the complete absence or defective character of the calcification of the cartilage, an abnormal amount of osteoid production, irregular invasion of the cartilage, and the production of a rachitic intermediary zone.

These investigators believe that rickets is produced by an unfavorable balance in the calcium and phosphate ratio, together with a deficiency of an unknown organic factor. When a diet is given which is high in calcium and low in phosphorus, and lacking the substance exerting an anti-rachitic effect, rickets is produced which resembles in every respect that found in human beings. When the phosphorus is high and the calcium low, the above picture is obtained. This differs in only a few and relatively non-essential details from that of the rickets which is generally agreed upon to be genuine. Since this type of lesion can be neither osteoporosis, nor osteosclerosis, and since in every clinical respect the disease is indistinguishable from rickets, Shipley, Park, McCollum and Simmonds (13) believe that there are two main types of rickets, the second being characterized by the above picture.

Lehnerdt particularly mentions the large amount of osteoid,

the intrusion of the osteoid network into the marrow cavity and its resemblance to Wegner's phosphorus sclerosis. He asserts that the sclerosis induced by strontium feeding is identical with that caused by feeding elemental phosphorus, and that it has nothing to do with rickets. It seems that the condition induced by Lehnerdt rather than being "strontium sclerosis" may possibly have been this second type of rickets. Since practically this same picture may be obtained without the addition of strontium, the question naturally arises as to whether the strontium had any specific effect on the bones.

The bones of Lehnerdt's experimental animals were examined by Stoeltzner (14). Her results are summarized in the following table.

	Gesamter Knochen		
	$CaCO_3$+$SrCO_3$	$CaCO_3$	$SrCO_3$
Kalkarm mit $Sr_3(PO_4)_2$ (trachtige Hundin)	55.58	50.74	2.17
Junges A u. B.	44.51	37.10	6.41
Junges C	47.34	36.29	6.08
Rachitischer Knochen		32.18	
Normaler Knochen		57.29	

With the diversified and conflicting data before us, and in light of improved feeding and chemical technique, it seemed quite worth while to investigate the specific effect of strontium on the animal body, particularly on the skeletal system. In order to do this three series of experiments, - chemical, physiological, and histological, have been planned, which are intended to answer the following questions.

Series I.

1. Is strontium deposited in the bones of the body?
2. At what rate is strontium deposited, and does this deposition reach a limit.
3. Is strontium retained in the bone permanently, or is it rejected when the animal receives normal calcium-containing food?
4. Does the age of the animal affect this deposition?
5. Is strontium deposited when an animal is receiving an optimal amount of bone-forming minerals?
6. Through what paths does strontium enter the skeletal system?

Series II.

1. Does strontium have a specific physiological effect on the animal body?

Series III.

1. Does strontium have a specific effect on the histology of the bone?

2. Are the changes in the bones slowly or quickly produced?

3. Are the changes produced in the bones permanent?

Order of Experiment.

In order to answer the question of these series, experiments were carried out as follows:

Series I.

1. Two and two-tenths per cent of strontium was added to a ration known to be satisfactory in every respect except that it was low in calcium. This was fed to five rats until they died. The large bones were removed and quantitative analyses for calcium and strontium made.

2. (a) Young animals were fed the above ration, and killed at intervals of three days. The last animal to be killed in this series received the ration for thirty-one days. The bones were analysed for calcium and strontium.

   (b) Young animals received the ration for one, two, three, and four days respectively. The bones were analysed for calcium and strontium.

(c) Two pregnant rats were on the above diet for thirteen and twenty-one days respectively. One mother was killed and four young removed from the uterus. The second mother was killed and seven young taken from the uterus. In each case the ash was examined for strontium and calcium.

(d) Another was on the above diet, to which 1.5 per cent calcium carbonate had been added for ten days prior to delivery. The animal was killed and seven young removed from the uterus and ashed. The ash was examined for strontium and calcium.

Series II.

1. Four different groups of animals were maintained on four diets which were (1) satisfactory, (2) satisfactory except for a deficiency of calcium, (3) satisfactory except for a deficiency of calcium and an addition of strontium, and (4) satisfactory except for an addition of strontium.

The general appearance, posture, growth curves, size, length of life, and appearance of organs, bones, teeth, and types of lesions were studied and compared.

Series III.

1. A comparative study was made of histological sections from the tibia of animals receiving four diets. The first of these was satisfactory in every respect. The remaining were satisfactory except (1) a deficiency of calcium, (2) a deficiency of calcium plus the addition of strontium, and (3) the addition of strontium.

2. Sections from the rats which were maintained on above diets and killed at four day intervals were studied.

3. Sections were studied from the rats which had received the low calcium plus strontium carbonate diet for thirty-one days and then maintained on a normal diet. The animals were killed

at successive periods of two weeks, the last one studied having received the normal diet for twelve weeks.

Chemical Procedure.

Since this work is based largely on analytical data, it seems proper that the methods employed should be stated in some detail. No satisfactory method for the complete analysis of bone ash containing strontium is to be found in text-books. The problem is one of peculiar difficulty because of the presence of phosphates and the high content of calcium with the magnesium and strontium.

McCrudden's method for the determination of calcium and magnesium in the presence of phosphates was, with some modifications, followed. Strontium was separated from the calcium and magnesium according to Treadwell and Hall (15). The procedure follows:

Bone sufficient to give about one gram of ash was used. The ash was dissolved in concentrated hydrochloric acid, and the

volume made up to about 500 cc. One drop of alizarine red was added, the solution made just alkaline with ammonium hydroxide, then just acid with hydrochloric acid. Twenty drops of concentrated hydrochloric acid and 1.5 gms. ammonium chloride were added, the solution was brought to boiling, and about 1.5 gms. of oxalic acid (or 2.5 times enough to precipitate all calcium present) were added with vigorous stirring. After cooling to room temperature the solution was neutralized to congo red with 20 per cent sodium acetate (about 20 cc), and allowed to stand over night. The precipitate was then filtered off and ignited. The ignited precipitate was dissolved in nitric acid, placed in a special lithium apparatus flask (according to Treadwell and Hall) and carefully evaporated to dryness before placing in an oil bath. It was dried in oil bath at 140° C, with a current of warm dry air passing over it for 1 1/2 hours. This precipitate contains the calcium and the greater part of the strontium. About 10 cc. of absolute alcohol were added to the precipitate, and the flask shaken at intervals for one hour, then 10 cc. of anhydrous ether were added and the mixture allowed to stand over night. The calcium nitrate is dissolved and the strontium nitrate remains as a precipitate by this procedure. The strontium precipitate was filtered off, dissolved in warm water, and precipitated as strontium sulphate by the addition of a small amount of dilute sulphuric acid.

An equal volume of alcohol was added and the mixture allowed to stand over night. It was then filtered and the filtrate washed first with fifty per cent alcohol containing a few drops of sulphuric acid, then finally until free from sulphate with ninety-five per cent alcohol. The calcium contained in the absolute alcohol-ether filtrate was precipitated as the oxalate, ignited, and weighed as calcium oxide.

The filtrate from which the calcium and strontium oxalates were first separated was evaporated to a small volume, digested with nitric acid to remove the oxalate, and the strontium in the solution was precipitated as strontium sulphate by the same procedure as that described above. The amount obtained by this determination was added to that obtained for strontium in the preceding determination.

It is very necessary in this method of analysis to use absolute alcohol and anhydrous ether for the separation of the strontium and calcium nitrates. Very small amounts of water in one or the other of these reagents will dissolve the strontium nitrate.

It seems very likely that this may have been the cause for Wildt's not obtaining any strontium by his analyses. In his discussion

of his method he omits any statement to the effect that he used absolute alcohol and ether.

The possible errors in the separation of the calcium from the strontium in plant ash are discussed by Robinson, Steinkoening and Miller (16) of the United States Department of Agriculture. They say:

"The determination of strontium in plant ash is not satisfactory. A large amount of calcium is always present, a fact which necessitates a large volume in which the precipitation is to be made. It seems probable that a considerable portion of the small amount of strontium present escapes precipitation in both the sulphate and oxalate separations. Further, some strontium nitrate is probably dissolved by the comparatively large amount of absolute ether-alcohol which must be used to dissolve the calcium nitrate and completely wash it from the strontium nitrate. An error in the other direction arises through the difficulty of washing the strontium nitrate free from calcium salts."

Thus it seems that the possibilities for error in the determination of strontium are much greater for too low rather than for too high results.

Demonstration of the Deposition of Strontium in the Bones

In order to determine if strontium is deposited in the bones, five rats were fed the following ration until they died. This ration shall hereafter be designated as Ration I.

Ration I.

	Per cent
Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
$SrCO_3$	2.2
Butter fat	3.0
Dextrin	62.8

The bodies were boiled for three hours, after which the flesh was easily removed from the bones. When the "percentage of ash" determination was made the bones were weighed, after being dried in an oven heated to 100° C for six hours. The bones were then ashed in an electric muffle heated to redness for one hour, and the residue weighed. The chemical analyses were made on the ash, and percentages calculated in terms of this constituent. The results of this work are tabulated in Table I.

Per cent of Calcium and Strontium Obtained from Bones of Rats on a Diet Containing 2.2 Per cent of Strontium Carbonate. (Ration I)

Days on diet before death	Calcium in ash	Strontium in ash
24	32.05	7.99
24	31.78	7.98
53	31.68	7.25
65	31.33	7.48
62	31.61	7.46

These results seem to indicate that death occurs when the strontium in the body reaches a limit.

Rate of Deposition of Strontium.

In order to determine the rate at which strontium is deposited, young animals were fed on Ration I, and killed at three day intervals. These results are tabulated in Table II.

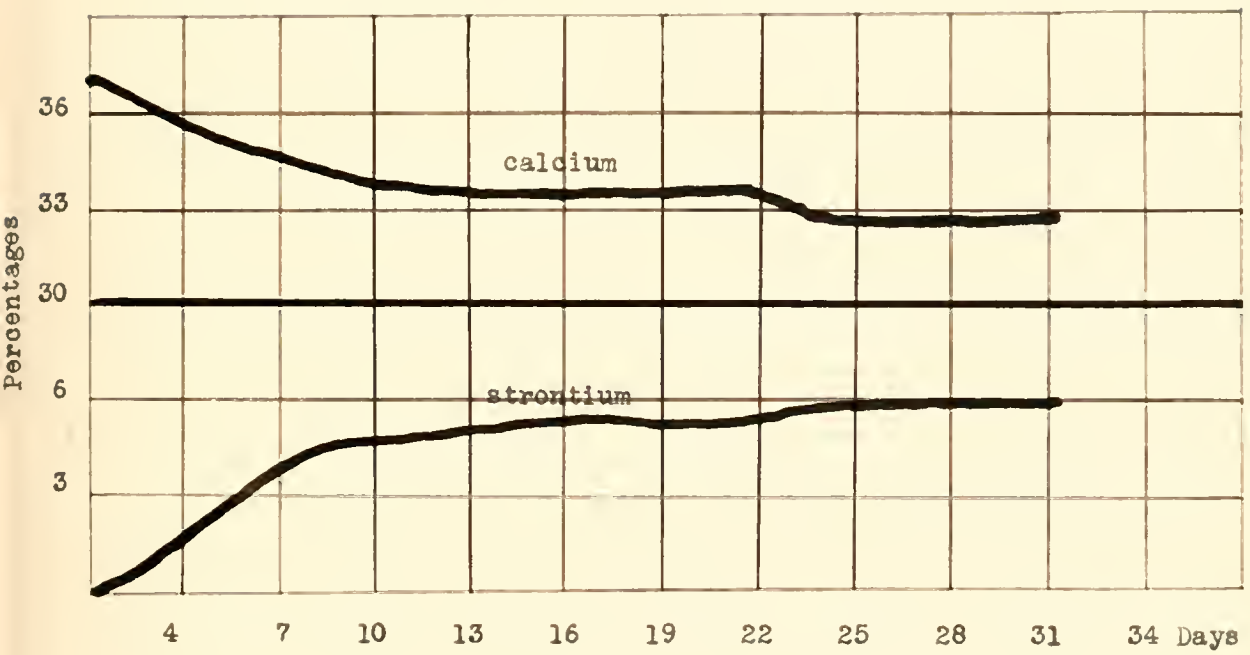
Table II

Effect on Composition of the Bones of Young Rats Receiving Ration I

Days on diet	Weight when put on diet	Weight when killed	Percentage calcium in ash	Percentage strontium in ash
0		80	36.63	0
4	89	85		2.61
7	63	59		4.04
10	92	79	33.84	4.41
13	102	115	33.49	4.50
16	101	110		5.25
19	100	126	33.28	5.10
22	96	124	32.34	5.35
25	93	116		Lost
28	96	102		5.67
31	81	92	32.86	5.97

Several interesting facts are to be observed in this table. The strontium is deposited very rapidly and regularly, and as the strontium increases the calcium decreases. The total amount of ash remains essentially unchanged. This is graphically shown in the following curves.

Curves Showing Increase of Strontium and Decrease of Calcium in the Ash of the Bones of Rats Receiving Ration I.



Permanence of Strontium Deposition

To determine if strontium remains in the bone permanently a group of young rats was given Ration I for thirty-one days. After that period they were placed on a ration consisting of

Ration IV

	Per cent
Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
$CaCO_3$	1.5
Butter fat	3.0
Dextrin	63.5

This ration we shall call Ration IV. So far as can be determined it is satisfactory in all respects for the nutrition of the rat. It contains the optimum amounts of mineral constituents, proteins of good quality, and the unknown accessory factors. It is to be observed that the only difference between Ration I and Ration IV is that the strontium carbonate of Ration I is replaced here by calcium carbonate, the small difference in the weights of these being made up by dextrin. When molecular weights are taken into consideration it requires 2.2 gms. of strontium carbonate to give the same number of molecules as 1.5 gms. of calcium carbonate.

Chemical analyses were made of the bones from two rats,

one which had been on Ration IV (strontium-free, calcium-containing diet) for eight weeks, and one which had been on the ration for fourteen weeks.

Table III

Effect on Composition of the Bones of Rats which had received Ration I for Thirty-one Days, then had been given Ration IV.

Weeks on Ration IV	Percentage ash	Percentage calcium	Percentage strontium
8	63.1	36.25	1.50
14	66.4	37.03	1.03

The results indicate that strontium is thrown out of the bone, but that at least the last portions are gotton rid of slowly. Observe that the percentage of ash and of calcium have reached the normal in the bones of the rat receiving the satisfactory diet for fourteen weeks.

The Effect of the Age of the Animal on Strontium Deposition.

In order to determine if the bones of mature animals were affected by strontium to the same degree as the young ones, rats weighing between 150-180 grams were fed Ration I. They were killed at four day intervals. The results are tabulated below.

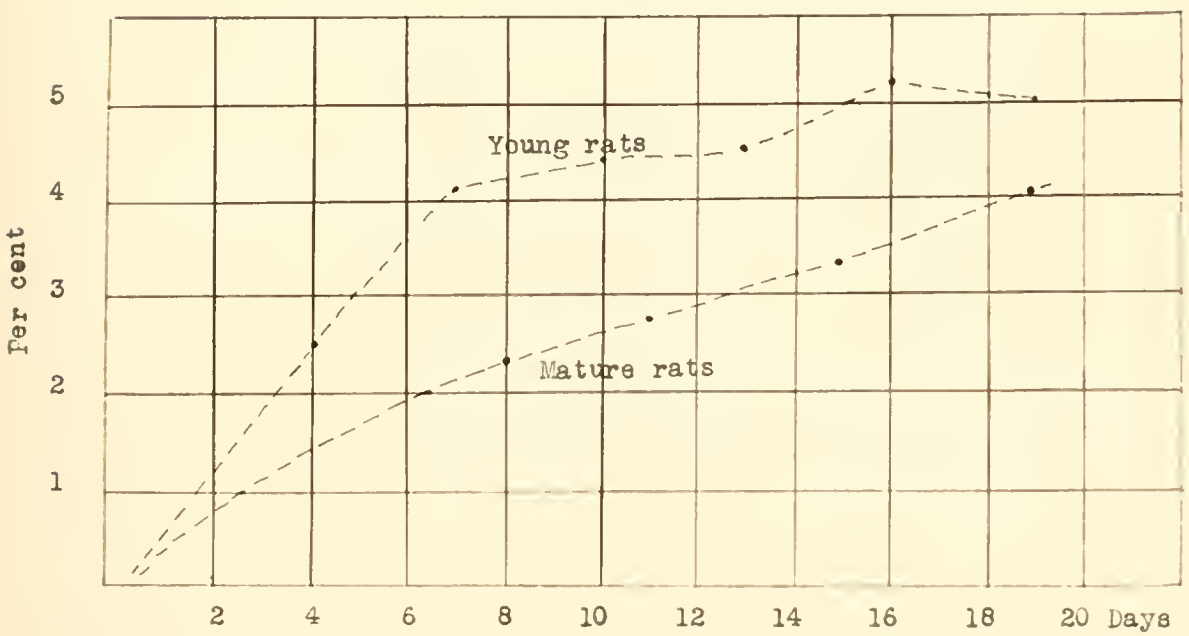
Table IV

Effect on Composition of Bones of Grown Rats by Feeding a Diet Low in Calcium, High in Phosphorus, and Containing 2.2 Per cent of Strontium Carbonate (Ration I)

	Days on diet	Percentage of ash	Percentage of calcium in ash	Percentage of strontium in ash
	4	65.2	----	1.33
	8	65.7	----	2.28
	11	62.1	34.09	2.76
	15	63.5	33.52	3.40
	19	62.8	34.35	4.07
Normal bone (stock animals)	10 mos. old	66.1	37.7	0.0

Again the strontium of the ash was increased and the calcium decreased as the feeding continued, but these changes did not take place so rapidly as in the younger animals. This might be expected, since, in general, the young and rapidly metabolising tissue is more susceptible to a change of condition of any sort than the mature one. The curves afford a graphic comparison of the rates of deposition of the strontium.

Curves Showing the Rate of Deposition of Strontium in the Bones of Young and Mature Rats.



Since such a high percentage of strontium was found in the bones of the rats which had received strontium for only four days, it was thought that it would be of interest to determine the amounts deposited in one, two, three, and four days respectively. Rats weighing between 100 and 130 gms. were used. The following results were obtained.

Table V

Percentage of Calcium and Strontium found in the Bones of Rats fed Ration I for Short Periods of Time

Days on diet	Percentage of ash	Percentage of calcium	Percentage of strontium
1	63.96	36.76	0.67
2	64.06	36.68	0.78
3	60.22	36.31	0.98
4	61.79	36.38	1.21

Effect of Strontium Feeding when the Animal is Receiving an Optimal Amount of Bone-forming Minerals

In order to determine if Lehnerdt's suggestion that strontium might not be deposited in the bone if a sufficient amount of calcium was supplied in the diet, 2.2 per cent of the dextrin in Ration IV was replaced by 2.2 per cent of strontium carbonate, as follows:

Ration II

Liver	20.0
Casein	10.0
NaCl	1.0
$CaCO_3$	1.5
KCl	1.0
$SrCO_3$	2.2
Dextrin	61.3
Butter fat	3.0

This ration was fed to mature rats, and the rats killed and the ash analysed exactly as in the preceding experiment. The data in Table VI were obtained.

Table VI

Effect on Composition of the Bones of Mature Rats Receiving a Diet Containing 1.5 per cent of Calcium Carbonate and 2.2 per cent Strontium Carbonate (Ration II)

Days on diet	Weight when placed on diet	Weight when killed	Percentage calcium in ash	Percentage strontium in ash
0		150	36.32	0.
5	148	141	34.13	1.76
9	162	155	34.60	2.19
13	167	186	35.79	2.17
17	183	216	34.57	4.04
21	178	218	34.98	4.90
25	170	194	35.76	3.25

Several interesting facts are evident from these data. First, Lehnerdt's premise concerning the effect of sufficient bone building minerals was wrong. The presence of a sufficient amount of calcium in the diet does not inhibit the deposition of strontium. The rate of deposition does not seem to be uniform in all animals,

but, in general, it appears to be about the same as in the mature animals receiving the low calcium diet (Ration I). On the other hand, the calcium makes a small drop in the first five days, but thereafter in g neral increases slowly toward the normal. The data would seem strongly to suggest the conclusion that strontium behaves independently of calcium as a constituent of the bone, and does not replace it physiologically as a bone-building element.

The Paths through which Strontium may enter the Body

In order to determine if strontium could enter the body through the placenta and through the milk of the mother, as well as by way of the alimentary tract, two series of experiments were carried out.

In the first, nursing rats of mothers receiving the strontium-carbonate diet (Ration I) were killed at varying periods and the entire bodies ashed. The amounts of strontium found in

rats nursed not more than four to six hours was 3.01 per cent. The mother had been on the strontium carbonate diet four days. In rats which had nursed four and nine days respectively, the mothers having had strontium for two days before the birth of the young, the strontium content amounted to 4.15 and 5.74 per cent respectively of the ash of their bodies.

The ash of young rats five days old, whose mothers had had no strontium, contained none of this element. These results are shown in the table given below.

Table VII

Rate of Deposition of Strontium in the Bodies of Young Rats Which Secured the Strontium through the Milk of the Mother

No. of rats.	Age of young. Days	Time of feeding strontium to mother before young were born. Days	Time of feeding strontium to mother after young were born. Days	Percentage of strontium in ash of young.
6	5	0	0	0
4	4	2	4	4.15
3	9	2	9	5.75
8	4-6 hrs	4	4 hrs	3.01

The data show conclusively that the milk of a mother receiving strontium contains this substance, and the young can receive it through this path.

To determine if strontium can be transmitted through the placenta, pregnant rats receiving Rations I and II, and as controls, rats receiving Ration IV, were killed, the young were removed from the uteri, the bodies ashed, and analyses made for strontium and calcium. It was found that the ash of the young from mothers receiving the strontium diet contained 3.34-3.54 percent of calcium and 2.34-2.88 per cent of strontium. The ash from the young of the mother receiving the calcium and strontium containing diets contained 10.32 per cent of calcium and 1.91 per cent of strontium. The ash from the young of mothers receiving the normal ration contained 9.9 - 13.9 per cent calcium and no strontium. The results are most easily compared from Table VIII.

Table VIII

Percentage of Strontium and Calcium found in the Bodies of Unborn Rate whose Mothers had received Rations I, II, and IV.

No. of days pregnant mother received ration	Ration	Number of young	Percentage calcium	Percentage strontium	Note
10	II containing strontium and calcium	7	10.32	1.91	Pregnant when put on ration
13	I containing strontium	9	3.54	2.34	Pregnant when put on ration
21	I containing strontium	7	3.34	2.88	Entire gestation period
Maintained on ration	IV containing calcium	9	9.9	0	Gestation period about two-thirds completed
Maintained on ration	IV containing calcium	7	13.90	0	Gestation period about completed

The most striking figures in this table are those for the rats whose mothers had received Ration I. It is to be observed that the amount of calcium is very low, and the strontium relatively high. The percentage of calcium in the ash of the rats whose mothers had received the calcium and strontium diet was apparently close to normal. It will be seen from the figures of the normal rats that the calcium of the ash increases as the gestation period progresses. The data show that strontium can be transmitted through the placenta to the body of the unborn rat.

To summarize very briefly the results of the preceding part of the experiment, we may say that strontium enters the body through the placenta, the milk of the mother, and the alimentary tract. Its entrance is not inhibited by the presence of calcium in the diet, but it enters the body of a mature rat more slowly than a young one. Death appears to intervene when the strontium has reached a maximum

limit of about 8 per cent; but if the animal is given an optimal non-strontium containing diet before the harmful effects of the strontium have proceeded to this stage the element is slowly discarded by the animal body.

Series II

The Specific Physiological Effects of Strontium on the Animal Body.

In order to determine if strontium had any specific physiological effects on the animal body, observations were made on the growth, appearance of the animals, peculiarities of locomotion, quality of the teeth and bones, and the appearance of the internal organs as seen at autopsy. The animals used for chemical analyses were observed and studied, but since these rats lived a comparatively short time, four additional groups of about the same age and weight were selected. Three of these groups were fed Rations I, II, and IV

respectively, and the fourth received a ration as follows, which shall be called Ration III.

Ration III

Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
Dextrin	65.0
Butter fat	3.0

This ration is similar in every respect to the ones previously described, except that it lacks both calcium and strontium. It is the same ration described by McCollum, Simmonds, Shipley, and Park (17), and called by them Ration 2581. The animals of these four groups were observed until they died from the effects of their defective rations or until the experiment was terminated.

General Appearance and Behavior of Rats fed Ration I

The general behavior of the animals receiving Ration I was most striking and characteristic. After about ten days or two weeks,

depending on the age of the animal, a slight palsied motion together with a "leap frog" position was observed. This condition became steadily worse for about two weeks, when muscular coordination was very difficult and the palsied and nervous movements constant and very severe. After that time, adaption seemed to set in and, although the posture became increasingly worse, that is, the animal's hind legs gave the appearance of growing longer and longer, throwing the rump higher than the head, and walking was accomplished with a paddling motion, the nervous and palsied tremors were only slightly apparent. After about seven weeks the animals walked with a stagger, frequently falling. This staggering and falling increased in severity until death. From about the eighth week they spared themselves all the movements possible. Also at this time they frequently cried if handled. Between the twelfth and thirteenth weeks the palsied motion again appeared very markedly, increasing in severity until just before

death. At that time the animals appeared to be in a partial state of coma, moving only very sluggishly or not at all when touched or handled.

After the beginning of the strontium feeding the animals grew little or not at all. (See curve). When, in a few cases after a period of thirty-one days of strontium administration, this element was omitted and replaced by calcium, growth followed at a rapid rate. Locomotion became normal in about eighteen days. The strontium fed rats showed some roughness of coat, but this condition was not extreme.

Findings at Autopsy of Animals fed Ration I

Autopsy showed several interesting features. These are tabulated in the adjoining table and need but a brief comment on certain more prominent features in which the experimental animals differed from the normal. The thorax was flattened from side to

side. There were no deposits of fat in the body, and the musculature was very much wasted. The gonads and thymus were atrophic. The adrenals were hypertrophied, in many instances to an extreme degree.

Effect on Bones and Teeth of Feeding Ration I.

The bones were found to be very cartilaginous and easily cut with a knife, were yellowish in color and stained in places with haemoglobin. They were of poor quality. The skull could not be removed from the soft tissues after boiling without its fragmentation. The epiphyses separated from the long bones, and were considerably enlarged. The ends of the bones turned almost black on drying. This appears to be the result of some effect of the strontium on the blood, but the explanation is not clear. The surface of the bones presented a very glossy appearance, which was so pronounced as to attract attention at once.

The teeth were evidently affected adversely by strontium feeding. The incisors were colorless, whereas in the normal rat they are a bright orange on the anterior surface. The pulps were exposed in these persistently growing teeth, and the efforts of the animal at attrition had been so effective that the upper incisors were cut too short. In the case of animals which were fed strontium for thirteen to fourteen weeks the incisors were practically disintegrated. In one animal there were no protruding upper incisors, but the roots lay buried in the maxillae. In most animals the mandible was perforated at the base of the incisor. This tooth was so long that it extends below and behind the molars, and to the bases of the condyles. The cortex of the mandibles, as in most of the other bones, appeared hard and well impregnated with mineral salts, and it seems remarkable that this perforation at the base of the incisor roots should have taken place.

The molars in young rats which were fed strontium during thirty days were somewhat adversely affected. In some animals one or more molars were missing, and in all, the alveolar processes were somewhat poorly developed around the necks of the teeth. No carious lesions were found.

Changes in the Bones when Strontium is Replaced by Calcium

As already stated, a period of thirty-one days of strontium feeding was sufficient to profoundly damage the bones. They were exceedingly cartilaginous, with enlarged epiphyses, and wide areas between the diaphyses and the epiphyses. The symphyses were all very distinct, especially in the pelvic bones, and they presented the appearance of very young bones, the parts of which were not yet united. The skull disintegrated at the sutures. The bones were dark and hemorrhagic. The damage to the teeth was more marked in the persistently growing incisors than in the finished molars.

After the strontium in the diet had been replaced by an equivalent amount of calcium for a period of two weeks the bones were found to be exceedingly friable. The pelvic bones were, without exception, fractured. The large area of osteoid at the junction of the epiphysis and diaphysis was newly calcified and quite deformed. This area was exceedingly porous and enlarged. The scapulae were dark, porous, and hemorrhagic. The bones seemed to be entirely lacking in fat, and had a chalky appearance. In general, they showed considerable deformity. This deformity was very noticeable in the scapulae, which were bent almost to right angles, and also in the skulls. In the latter the median line was a curve. The teeth had no cavities, and all the molars were present. The incisors were light yellow and showed pulp exposures.

After four weeks on the diet in which calcium was made to replace strontium, the bones had grown decidedly in length. They

were somewhat greasy in appearance, and had become better calcified. The skull was asymmetric, the median line being a curve. The hemorrhages were pronounced only in the femora. The teeth were badly worn.

After six weeks from the condition described as resulting from feeding strontium for thirty-one days the bones were still somewhat porous. The long bones were somewhat greasy, but the flat bones were still chalky. The scapulae were cupped, but this condition seemed to be in process of correction as compared with those in rats which had received calcium for two weeks. There were still no cavities in the molars.

The bones from rats fed the strontium-free, calcium-containing diet for eight weeks were better calcified than those receiving the diet for six weeks. The skeleton was approaching the normal appearance. The median line of the skull was very crooked laterally, producing such a

bend in the maxillae that on the side having the lesser curvature the molars were crowded, one being badly impacted. This condition has never before been observed in the rat.

After ten weeks of recovery on the strontium-free diet the bones were well calcified. The color was essentially normal. The symphses in the pelvic bones were completely united and the bones were hard and smooth. No evidence of hemorrhage persisted. The incisors were now yellow in color on the anterior surfaces.

A remarkable observation made in connection with these experiments related to changes in the teeth. The molars remained sound during the period of strontium feeding, which covered thirty-one days, but rapidly disintegrated when the strontium in the diet was replaced by calcium. Caries-like lesions developed under these conditions in about ten weeks. The incisors, being persistently growing teeth in this species, had an opportunity to repair the damage which they had suffered as the result of the strontium feeding, and

rapidly regained an essentially normal structure, color, and appearance.

It has been mentioned already that on the replacement of strontium by calcium, the strontium contant of the bones rapidly fall from about 7 per cent to 7 1/2 per cent of their ash content. Our analytical data are not sufficiently complete to enable us to say just how much of the rapid loss of the strontium content is due to new growth of the bones, and how much is due to elimination of strontium and its replacement by calcium. Without doubt both factors are operating to produce the result observed. It is of special interest, therefore, to observe what appears to be almost destruction of the bones during the first four weeks after the strontium has been replaced by calcium in the diet. This appears to be a part of the general process of resolution of the skeletal tissues of the body. We shall see later that the changes in the histological structure of the bones under the conditions we have just discussed harmonize with the view

that the strontium-laden bones are rapidly resolved, their contents being mobilized for the purpose of elimination of the offending substance as soon as the strontium is left out of the food and calcium is supplied in liberal amounts.

Effect of Feeding a Diet Containing the Optimal Amount of Calcium Together with Two and Two-tenths Per cent of Strontium Carbonate

In order to see if the preceding pathological conditions were due to the lack of calcium in the diet, rather than to the presence of strontium, animals were fed a diet which contained an optimal amount of calcium and an equivalent amount of strontium (Ration II).

General Appearance and Behavior of Rats Fed Ration II

After rats had been on this diet for about three weeks, a slight paddling motion of the hind legs when they walked, and some lack of coordination of the movements, were observed. This practically disappeared by the end of the eighth week. After that

time the animals presented very little difference from the normals. Their coats were somewhat rough, and, although their bodies were not fat neither were they thin; they did not gain in weight at the rate of a normal rat, but they did gain slowly.

Appearance on Autopsy of Rats Fed Ration II.

The findings at autopsy were only very slightly different from those of a normal rat. However, the rats autopsied had received the ration only thirteen weeks and had not died from its effects. Under these conditions the only abnormalities presented were those in the teeth and spleen. The spleen was atrophied. The teeth will be disoussed under a separate heading.

Effect on Teeth and Bones of Rats Fed Ration II

When the diet contained both calcium and strontium the bones did not suffer as serious injury as they did when the diet contained a fairly liberal amount of strontium but was deficient in

calcium. Under the former circumstances the bones, during the early weeks of the experiment, showed some tendency to being porous and hemorrhagic. Large borders of cartilage surrounded the scapulae. There was no abnormal enlargement of the ends of the long bones. The sutures which unite the several parts of the pelvic bones at the socket remained visible, but the parts of the bone were firmly united. There was easily noticeable the glossy surface characteristic of the bones of animals fed strontium, but this was less marked than when the diet was deficient in calcium.

The teeth showed a tendency to lose color. The pulps were exposed in the upper incisors. Caries developed in the molars. The alveolar processes were not quite so well developed around the necks of the teeth as in the normal. The skulls remained symmetrical in all cases.

At the end of five weeks the bones of some of the animals

were darker and more hemorrhagic than before. They had no greasy appearance, but were rather dry and chalky in texture. The pelvic bones were fractured across the socket at the sutures which should fuse in early life. The incisors of these animals still retained a certain amount of color on their anterior surfaces.

At five to seven weeks from the beginning of the experiment, the bones of rats under the above experimental conditions were in the worst condition that they ever reached. After that time they began to return toward the normal in a manner which suggests that the body in some way habituates itself in the presence of strontium, and acquires a tolerance for it. This suggestion is also strengthened by the general physiological behavior of the rats. After the seventh or eighth week the awkwardness of movement and locomotion had practically disappeared, the animals appearing to be essentially normal. These statements

do not apply to the teeth. After thirteen weeks of feeding with both calcium and strontium the bones were approximately normal in external appearance. The teeth, however, were very bad at this time. The upper incisors had completely lost their color and were too much shortened by attrition. The lower incisors were overgrown and colorless. They showed no pulp exposures, but there was considerable hyperplasia. The molars were carious, and defects of the attaching tissues were severe.

A most interesting fact brought out by our observations is the relation of strontium feeding to the color of the incisors. Nothing is known concerning the cause of the presence of color in the teeth, but teeth of good quality are always colored. Strontium appears to cause the disappearance of this color entirely either when calcium is deficient in the diet or when it is provided. The incisor is a persistently growing tooth, being found normally at the rate of

about 2 mm. per week. It is most interesting that the color reappears in this tooth almost as soon as the strontium is removed from the diet.

The Effect of Feeding a Ration Low in Calcium and Containing no Strontium

Since all the severe and spectacular effects produced by the low calcium and strontium containing diets (Ration I) were not duplicated when a ration containing both strontium and calcium were fed, it seemed worth while to observe the effects produced by a diet defective only in its lack of calcium. (Ration III). It was reasoned that in this manner effects due to (1) the lack of calcium together with the presence of strontium, (2) the presence of strontium alone, and (3) the lack of calcium alone, might be classified, and attributed to a specific source or combination of sources.

Effect on General Appearance and Behavior of Animals Fed a Low Calcium Diet

The animals fed the low calcium diet (Ration III) were thin and undersized. The coats were rough and the hair irregular in length. They gained on an average about 20 gms. in weight when fed over a period of sixteen weeks. Young animals live but a short time on this ration. (17) The normal increase in weight of animals the age of the ones used in this experiment is about 80 gms. for sixteen weeks.

Appearance on Autopsy of Animals Fed a Low Calcium Diet

The bodies of the animals at autopsy showed thoraces somewhat flattened from side to side. The costochondral junctions were slightly enlarged. The musculature was very much wasted and there were no deposits of fat. The thymus and gonads were atrophied.

The spleen usually was greatly enlarged. The adrenals were very pale. The long bones cut easily.

Effect on the Teeth and Bones of Rats Fed Ration III

The bones were exceedingly chalky and porous. They showed little cartilage but the epiphyses of the long bones were enlarged. The shafts of the bones were slender. The skulls were brittle and showed a tendency to fracture along the sutures, which were not well knit together. The pelvic bones showed no fractures.

The teeth were carious and could easily be pulled from their sockets; attaching tissue defects were very marked. The incisors were normal in color, very brittle, and easy to extract. The upper ones were broken off, and the lower ones were over grown. The latter condition always occurs when defective attrition is at hand. Pulp exposure was present in every incisor.

A comparison of these animals with the preceding and with

the normals (rats fed Ration IV) are more easily made from the growth curves and tables which immediately follow.

The question may arise at this point as to whether the conditions met with when the calcium plus strontium containing diet was fed were in any way comparable to a diet containing two equivalent amounts of calcium, that is 3 per cent. Such diets have been fed in this laboratory. The animals were maintained in splendid condition, and the bones were excellent in quality. (Unpublished data). This strengthens the suggestion that strontium has effects on the animal body which are attributable to it alone, but these effects are greatly augmented if at the same time calcium is lacking.

These observations are interesting when compared with those of Meltzer and Auer (18). These investigators studied the effects of strontium as compared to calcium in its antagonistic action toward

Table IX

Table showing comparison of external characteristics and certain of the autopsy findings of rats fed Rations I, II, III and IV

Ration	Posture	Walk	Body size	Coat	Eyes	Thorax	Musculature	Fat	Costo-chondral junctions
I strontium	very abnormal	very abnormal	++	somewhat rough	normal	somewhat flat laterally	very wasted	none	somewhat enlarged
II strontium and calcium	slightly abnormal	slightly abnormal	+++	somewhat rough	normal	normal	not quite normal	some fat	normal
III no strontium or calcium	normal	normal	++	somewhat rough	normal	somewhat flat laterally	very wasted	none	slightly enlarged
IV calcium	normal	normal	++++	smooth soft	normal	normal	normal	normal	normal

Table X

Table showing comparison of internal organs of rats fed Rations I, II, III and IV

Ration	Intestinal tract	Thymus	Spleen	Gonods	Kidney	Adrenals	Lungs
I strontium	tendency toward congestion	atrophied	enlarged	atrophied	sometimes congested	often hyper-trophied	normal
II strontium and calcium	normal	somewhat small	normal	normal	normal	normal	normal
III no strontium or calcium	normal	atrophied	sometimes enlarged	very atrophied	normal	sometimes very pale	normal
IV calcium	normal	normal	normal	normal	normal	normal	normal

Ration	General texture	Quality	Fractures	Caries in teeth		Color	Condition of incisors	Defects
				molars	incisors			
I strontium	very soft and cartilaginous, easily cut	exceedingly thin, glossy when dissected and dried, some haemorrages	many	very few	always present	none	broken off or overgrown	enlarged epiphyses and ends of bones
II strontium * and calcium	hard, well ossified, practically normal	firm, white	none	few	present in upper, absent in lower	none	broken off or overgrown	normal
III no strontium or calcium	chalk like, brittle	porous, dark	many	sometimes present	present	normal	broken	ends of bones slightly enlarged
IV calcium	hard, well calcified	firm, white	many	sometimes present	none	normal	excellent	only occasionally small abnormalities

* after being on diet for some weeks

Chart I

The curves represent the increase in weight of two animals maintained on Ration I. This ration is satisfactory in all respects, containing the necessary mineral elements in optimal amounts, proteins of good quality, and the necessary vitamins. The animals looked well nourished, and produced and reared young to the weaning stage.

CHART 1, RATION IV

Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
$CaCO_3$	1.5
Dextrin	63.5
Butter fat	3.0

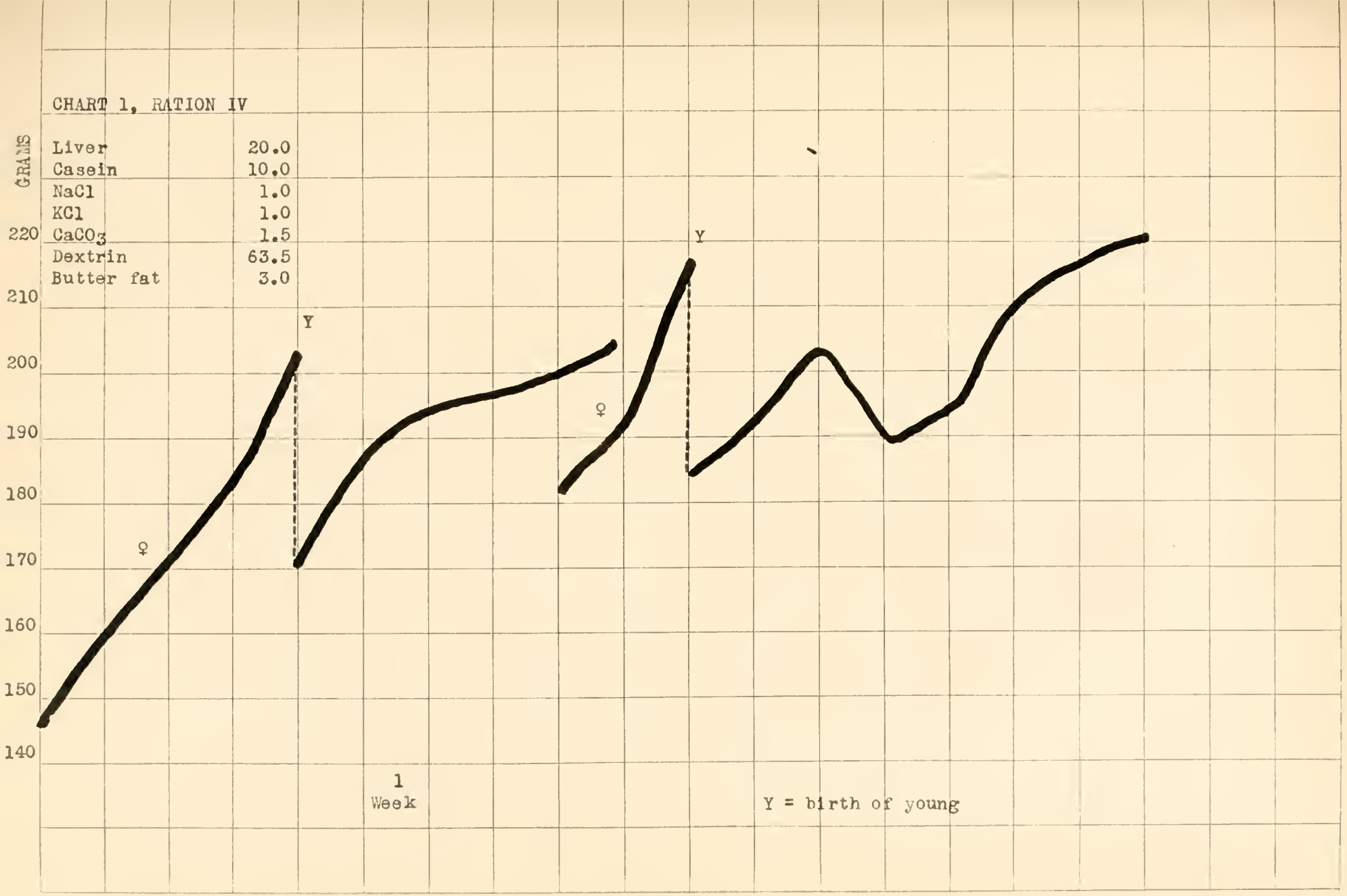


Chart 2

The curves of chart 2 show the effect of leaving calcium out of Ration IV. For a considerable period of time animals of the weight represented in the chart just about maintained their initial weight. Any gains that were made were very small as compared to those of the animals receiving Ration IV. These animals were of very low fertility, but if young were produced they were soon destroyed. Young animals died quickly on this ration.

CHART 2, RATION III

Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
Dextrin	65.0
Butter fat	5.0

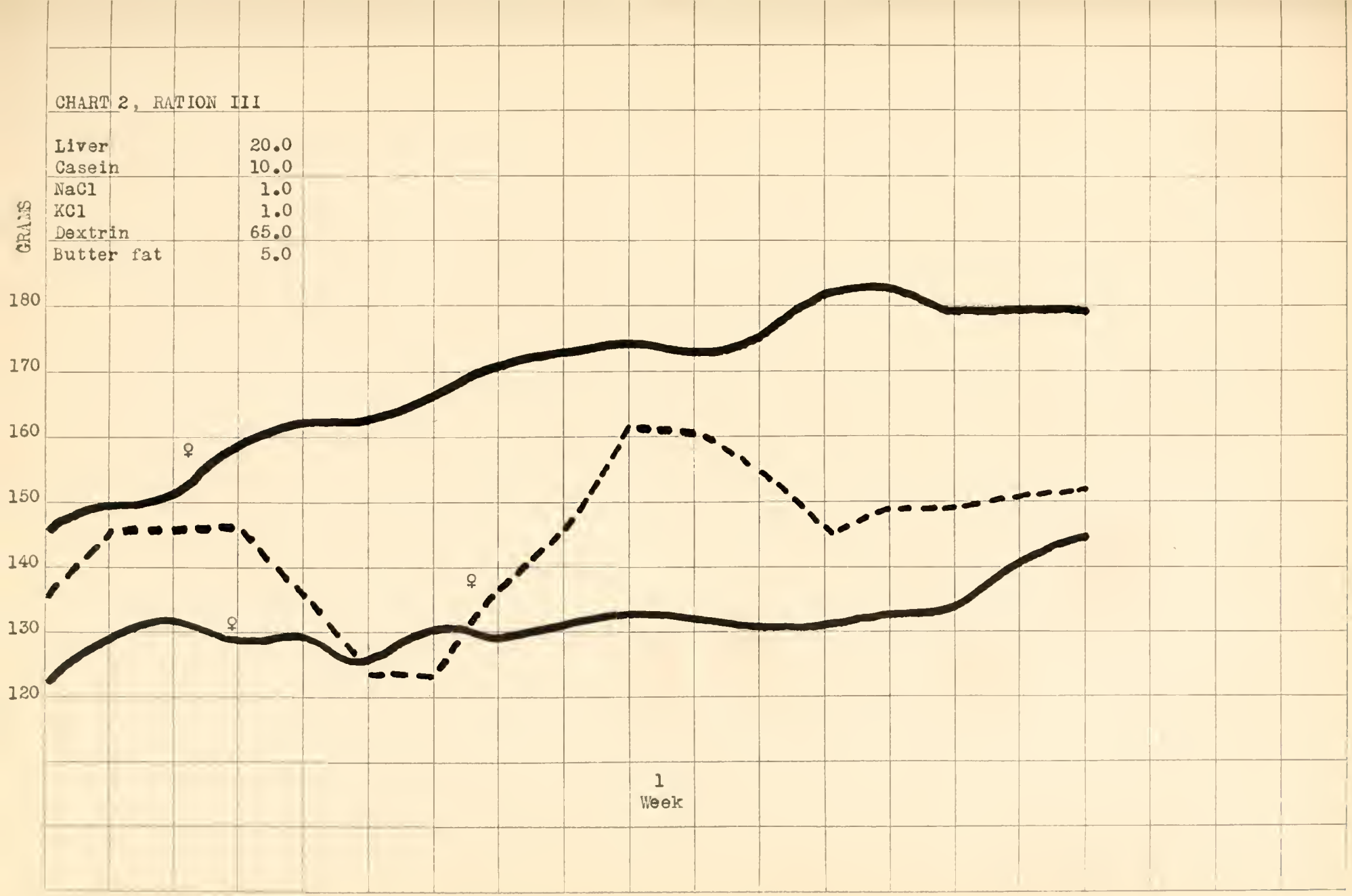


Chart 3

The curves of chart 3 show the effect of replacing the calcium of Ration IV by 2.2 per cent of strontium carbonate. For the first two weeks the animals lost weight rapidly. Then the body appeared to tolerate the strontium somewhat better and usually gained in weight for a number of weeks. If animals on this ration produced young, they soon destroyed them. The rats were thin and badly nourished.

It will be seen, when these curves are compared with those in chart 2, that strontium definitely shortens the span of life. All the animals on this ration died within seventeen weeks. None of those receiving the same ration, except that it contained no strontium, had died during the experimental period.

CHART 3, Ration I

Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
$SrCO_3$	2.2
Dextrin	62.8
Butter fat	3.0

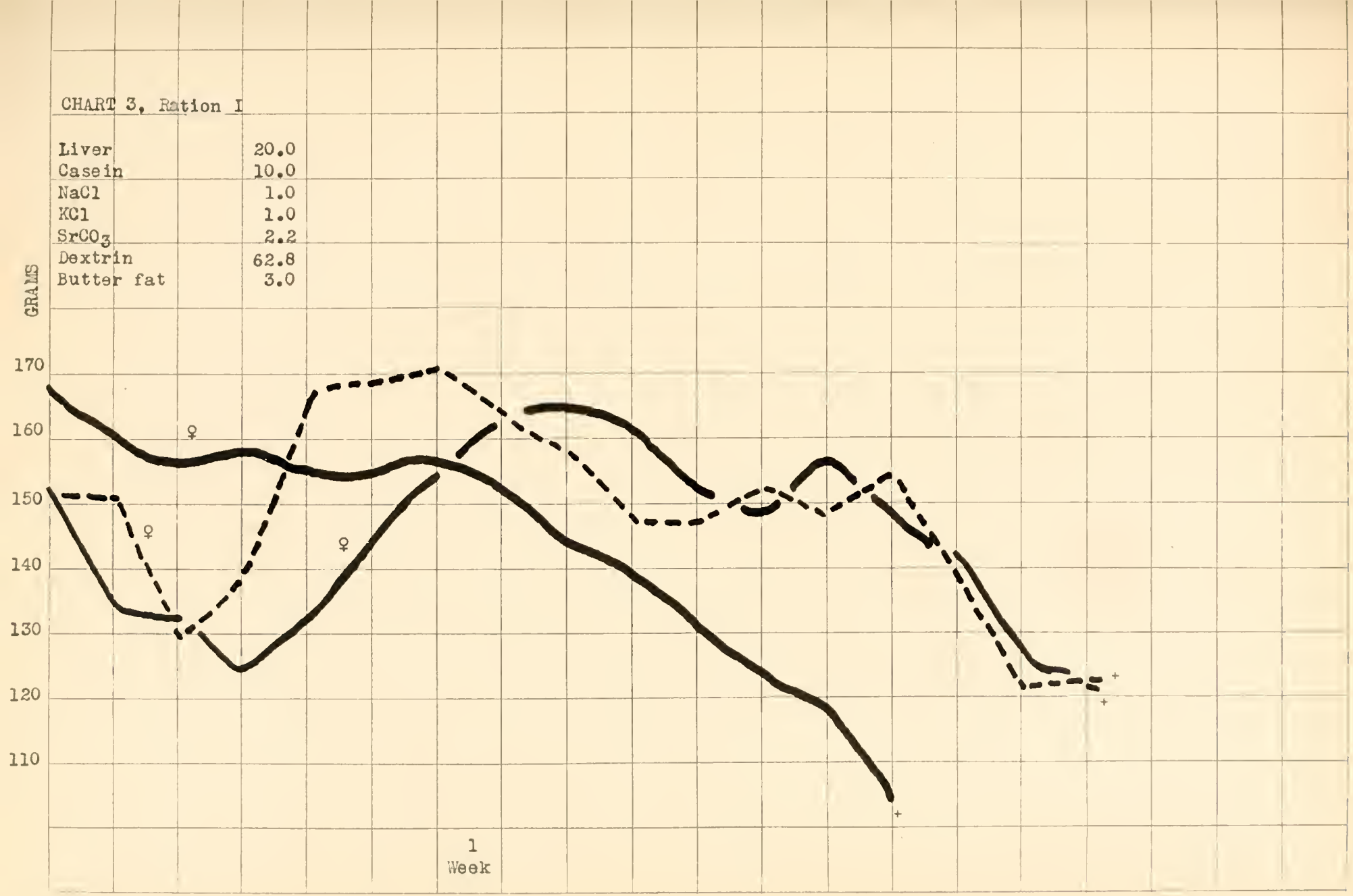


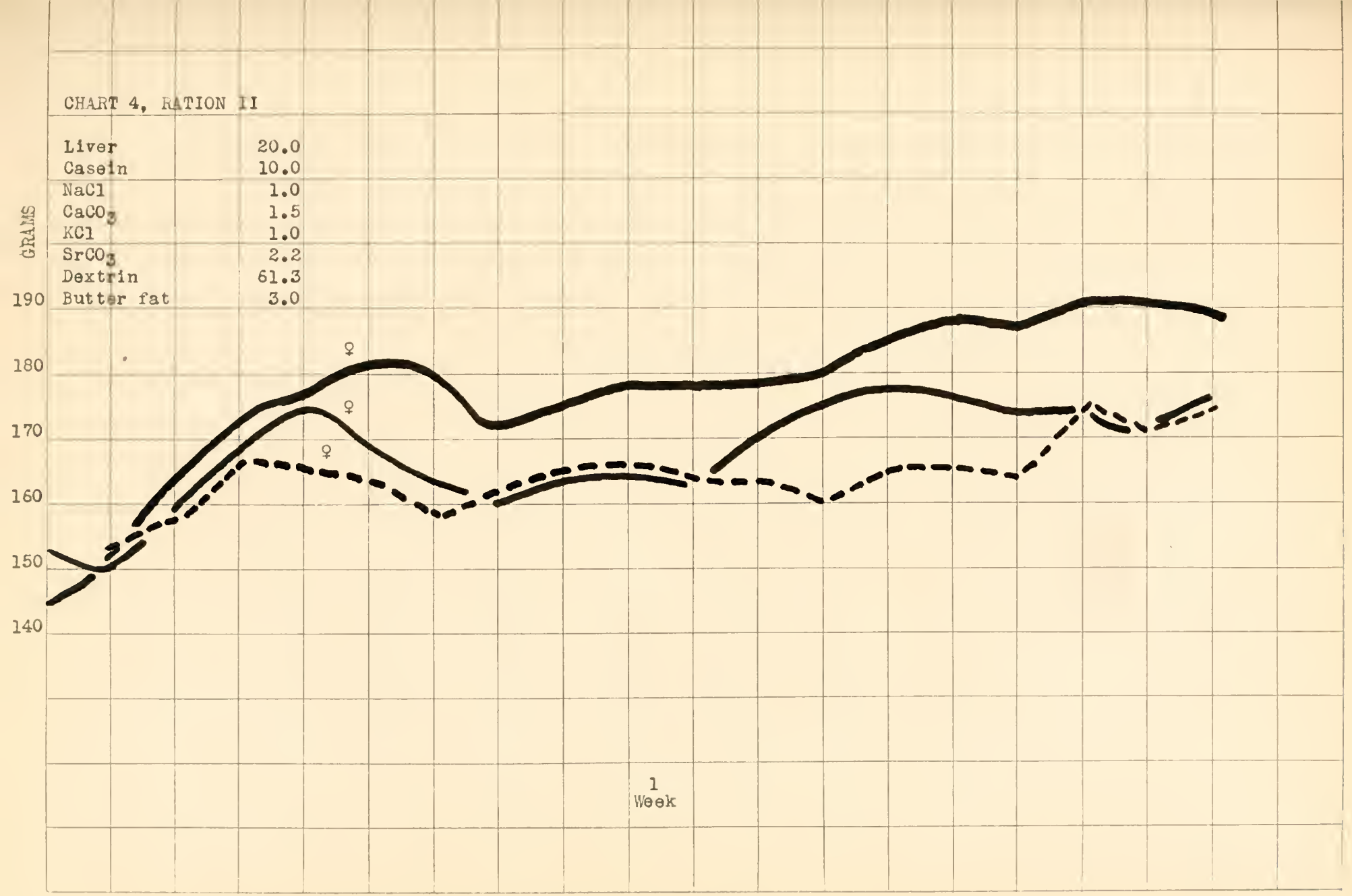
Chart 4

The curves of chart 4 show the effect of adding strontium to Ration IV. The animals grew slowly as compared to those receiving Ration IV, but they grew at about one-fourth of the optimal rate. (See curves of chart 1).

A comparison of these curves with those in chart 3 seems to indicate that calcium has a neutralizing effect on the toxic properties of strontium. In general the curves are more nearly normal than those of chart 2 (rats receiving neither strontium or calcium).

CHART 4, RATION II

Liver	20.0
Casein	10.0
NaCl	1.0
$CaCO_3$	1.5
KCl	1.0
$SrCO_3$	2.2
Dextrin	61.3
Butter fat	3.0



the anesthesia produced by the subcutaneous injection of magnesium chloride. They found that the action of strontium under these conditions was completely different from that of calcium, and that calcium was not antagonistic to the effect of strontium, but rather, if anything, increased them.

This certainly was not the case under the conditions imposed in these experiments. Here the calcium certainly did not augment the effect of the strontium. Rather, it appeared to have a neutralizing effect, particularly in the nervous reactions caused by the feeding. However, these results may not have been directly attributable to the action of the two elements as such, but occurred because calcium is needed by the animal body for normal metabolism, and a body provided with an optimal amount of this element is more able to resist the destructive action of an offending substance than one in which the calcium is very low. Therefore, to summarize the

results of these observations we can say that

Strontium produces, when calcium is lacking,a very marked lack of coordination in the animal body, and a peculiar and specific type of nervous reaction. The bones of such animals are often almost entirely uncalcified, being made up of cartilagenous tissue. These conditions are found to only a very slight degree when calcium as well as strontium is present in the diet.

Strontium, either with or without the presence of calcium, destroys or inhibits the production of pigment in the persistently growing tooth.

Strontium definitely shortens the span of live. Life is considerably lengthened if the animal received calcium at the same time that it receives strontium. We cannot yet say how much this increase may be.

A study of the specific effect of strontium on the bone follows:

Series III

An Histological Study of the Effects of Strontium on the Bones of Rats

In order that the influence of strontium on the histology of the bone may be easily understood, it is necessary first to understand the normal processes of growth in the developing bone. McCollum, in his book "The Newer Knowledge of Nutrition" (19) gives a brief and clear discussion of this subject and his description is quoted here.

"The growth of a bone in length is due to a definite sequence of events which transpire at the junction of the shaft of the bone (diaphysis) with its head (epiphysis). The shaft is composed of growing bone, the epiphysis of cartilage which is largely in a resting state. As the animal grows the cartilage of the epiphysis is converted into bone through (a) encroachment on it of the bony shaft and (b) through the expansion of one or more centers of ossification which form in it. The process of growth in length of the shaft is the same which results in the expansion of the centers of ossification in the epiphysis.

The cells of the resting cartilage have no particular

arrangement until they come within a short distance of the shaft or center of ossification. The cartilage cells then arrange themselves into columns parallel to the long axis of the shaft or the radii of the ossification center. This columnar zone is known as the "proliferative cartilage." Lime salts are deposited in the matrix separating the cells of the thin layer of cartilage in contact with the shaft. This is known as the zone of provisional calcification. Cartilage cells in this zone lie in a matrix which is like a calcified honey-comb. As the shaft grows tiny blood vessels sprout evenly from it and each vessel opens a single compartment in the calcified honey-comb. The cartilage cell is freed to degenerate or to become an osteoblast or bone-forming cell. Large numbers of osteoblasts follow the blood-vessel sprouts into the cartilage. These settle on the spicules of calcified matrix which remain after the cartilage cell capsules are opened. These spicules furnish cores about which the trabeculae of bone in the shaft are built. The osteoblasts on the spicules of calcified matrix bury themselves in a tissue known as osteoid which they manufacture. This receives a deposit of lime salts and becomes bone.

Growth in thickness of the shaft is accomplished by cells in a thick layer of connective tissue which surrounds the shaft and is called the periosteum. These connective-tissue cells are

transformed directly into osteoblasts and lay down bone in layers beneath the periosteum. During growth, and to a lesser extent after growth has ceased, the structure of a bone is subject to constant change to meet the requirements of exercise and changing conditions of stress and strain. Old bone is constantly being removed (resorption) and new bone is always being formed. In a normal, healthy bone these processes are always in a dynamic equilibrium."

The Specific Effect of Strontium on the Bone

That we might answer the question as to whether strontium exerted a specific effect on the histology of the bone, sections were made from the tibia of rats which had been maintained on Rations I, II, III, and IV. In this way a normal bone could be compared with one which was affected by the lack of calcium, the presence of strontium, and the lack of calcium together with the presence of strontium.

Microphotographs have been made of these four types of bones and are reproduced in Figures 1, 2, 3, and 4. They need to be but briefly described here.

The Histological Appearance of the Bones of Rats Fed a Satisfactory Diet (Figure I)

Bones from a rat which had received a diet in which the mineral content was satisfactorily adjusted, the proteins of good quality, and the necessary vitamins present, presented several features which were quite different from those of rats receiving the deficient diets.

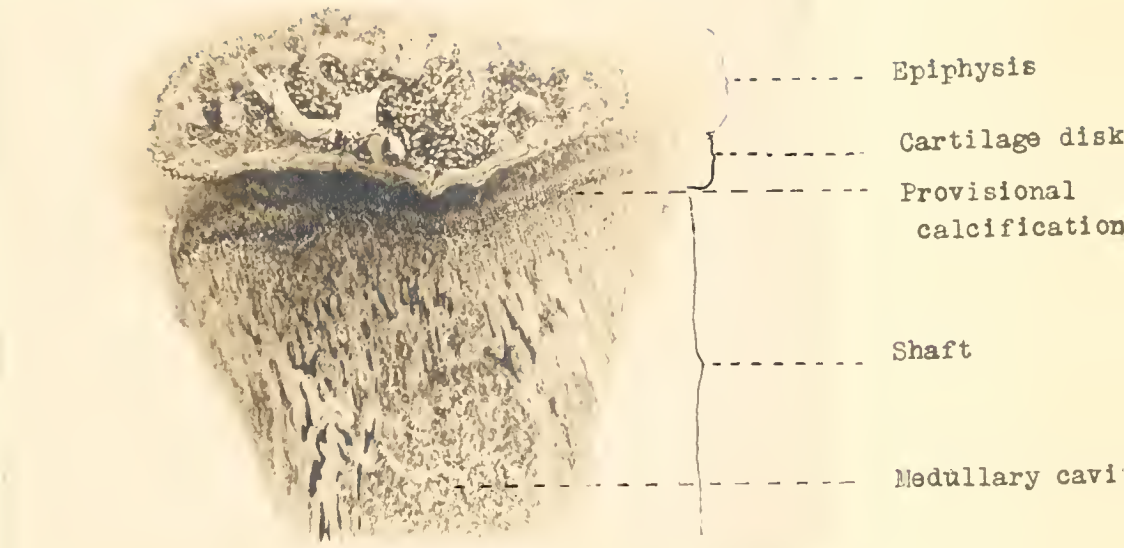
The proliferating cartilage disk in a young and growing bone was about twelve to sixteen cells deep.

The zone of provisional calcification presented a regular even line crossing the bone at right angles to the shaft. Under high power it appeared like a calcified honey-comb invaded by blood vessels and osteoblasts. It is in this region, as described by McCollum, that growth in the length of the bone takes place. The medullary cavity was sharply defined. The bone trabeculae were

Figure 1

Photomicrograph of a normal rat bone from an animal of the age of those employed for strontium feeding. For the purpose of comparing the experimental animals with the normal, the width of the disk of cartilage, and the proportion of it which exists in the swollen cell condition or vesicular stage, should be noted. Observe that the line of provisional calcification crosses the bone at right angles to its longitudinal axis and roughly parallel to the upper border of the proliferative zone. The magnification is not great enough to permit the honeycomb structure of the deposit being made out. The number of bone trabeculae and their orderly arrangement with the long axis of their sections parallel to the long axis of the shaft is characteristic of normal development. The photograph also shows the extent to which marrow elements appear in sections of the bone which is undergoing orderly development under suitable dietary conditions. The medullary cavity is sharply defined. The cortex, or dense layer of bone on the periphery of the shaft, is not shown in the photograph because of a re-touching of the negative at this point by the photographer. This is unfortunate in that it eliminated one of the characteristic features of the bone, but has no importance for the purpose for which the picture is reproduced here.

Fig. I


Epiphysis
Cartilage disk
Provisional calcification
Shaft
Medullary cavi

arranged in an orderly manner with their long axis parallel to the shaft.

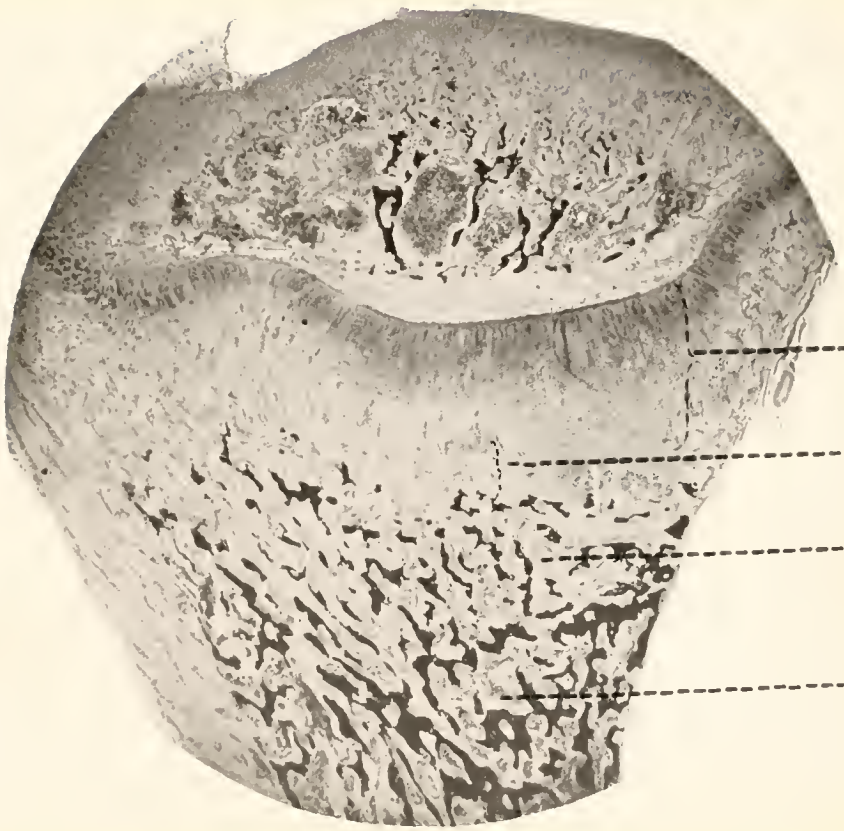
The Histological Appearance of the Bones of Rats fed A Diet Free from Strontium, and Very Poor in Calcium (Figure 2)

Under these conditions of diet the columnar arrangement of the cartilage cells was maintained. The cartilage disk was much wider than the normal, and there was as a rule no calcification in the proliferative zone, but occasionally traces of provisional calcification were seen. The metaphysis formed was very narrow. The cell cytoplasm lost its affinity for basic dyes. The number of bone trabeculae was very great, and these were slender and surrounded by wide borders of osteoid. They did not exhibit the regular arrangement seen in normal bones in which the trabeculae of bone tend to lie parallel to the length of the shaft, but instead formed a lattice-like structure. This was, however, more regular in its arrangement than

Figure 2

Photomicrograph of tibia of a rat which had been maintained on a low calcium diet. Observe that the cartilage disk is very much widened but that the trabeculae have retained their ordinary arrangement. The metaphysis is very narrow. The photograph does not show the many osteoblasts, blood vessels, and marrow elements which invade it. The confines of the medullary cavity have been entirely lost. The shaft is filled with the mesh-like spongiosa. The trabeculae of the bone are numerous; these have lost their normal longitudinal arrangement. Also, they are invested with thick borders of osteoid.

Fig. 2



the trabeculae seen in a rachitic metaphysis. We have reproduced here the picture which Shipley, Park, McCollum and Simmonds (13) call a second type of rickets, this second type differing from that which is generally accepted to be rickets only by a slightly greater degree of regularity of those elements which form rachitic metaphysis.

The Histological Appearance of the Bones of Animals Fed a Diet Deficient in Calcium but Containing Strontium

(Figure 3)

In comparison with the above described features of a bone of an animal which had ingested a satisfactory diet except that the calcium content was too low, the feeding of strontium led to an extraordinary stimulation of cartilage proliferation. This is shown in Figure 3. After several weeks the vesicular cartilage area was very wide, and the cartilage tended to persist, although with some

tendency to lose its characteristic staining reactions. Long tongues of cartilage interdigitated with osteoid tissue, which contained many blood vessels and marrow elements. This excessive formation of osteoid, and of cartilage proliferation, was not accompanied by any deposition of calcium salts. There were no trabeculae of bone in the neighborhood of the region of growth nor for a considerable distance down the shaft. The distinctly lattice-like arrangement of the spongiosa of bones of animals fed low oalcium was lost to a great degree when strontium was fed. The metaphysis more nearly approached that found in true rickets. Here again the strontium seems to have accentuated the effects caused by a low calcium diet. The production of osteoid, the width of the proliferating cartilage, and of the area of metaphysis, and the disorderliness of the elements of the metaphysis,are found to a greater degree than when the diet is deficient in calcium alone.

Figure 3

Photomicrograph of a bone from a rat which had received a low calcium, strontium-containing diet for thirty-one days.

Note the excessive width of the proliferating cartilage border. The light areas are filled with osteoid trabeculae, blood vessels, and marrow elements. These are intermingled in a somewhat disorderly fashion. Small irregular patches of provisional calcification persist.

Fig. 3

Proliferating cartilage
Metaphysis
Provisional calcificatio

The Effect on the Histology of the Bones of Feeding a Diet Containing in Addition to the Optimal Amount of Calcium, an Addition of Strontium Salt. (Figure 4)

At the end of a seven weeks period of feeding a ration containing both strontium and calcium the bone of the shaft was almost completely replaced by osteoid. The proliferating border of the cartilage disk was only a trifle wider than the normal, and the cartilage trabeculae retained their orderly arrangement. The metaphysis was very wide. In some areas the blood vessels and marrow elements and osteoid trabeculae intermingled in a most disorderly fashion. But in the greater part of this metaphysis these elements showed a more or less orderly arrangement, approaching the sclerotic picture seen in the low calcium bones.

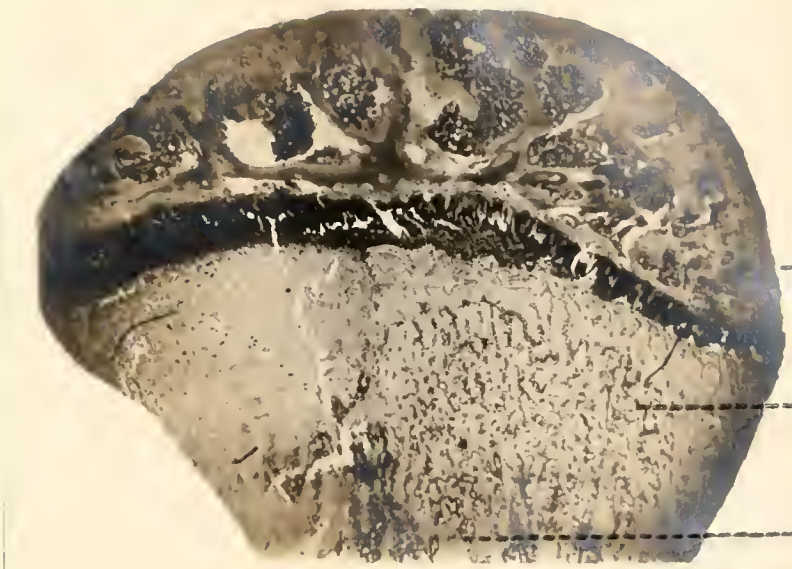
It is evident from the histological sections that when calcium and strontium are fed together there is a decided tendency for the former element to counteract the influence of the latter. The proliferation of cartilage is depressed, the calcium salts are

Figure 4

Microphotograph of a bone from a rat which had received a diet containing both strontium and calcium.

Observe that the proliferating cartilage disk is almost the same as that of the normal. The light area on the right, when seen under high power, is filled with trabeculae of osteoid, osteoblasts, marrow elements, and blood vessels intermingled in a disorderly manner. The metaphysis occupying the rest of the field is very similar to the one seen in Figure 2. The regular mesh-like arrangement of the osteoid trabeculae, with the interstices filled with marrow elements are seen in the picture. Areas of the marrow cavity remain unoccupied by the spongiosa.

Fig. 4


Provisional calcification
Proliferative cartilage dis
Metaphysis
Marrow cavities

deposited to some extent, and the entire picture of the finer structure of the bone is distinctly shifted in the direction of normal.

The Rapidity of the Effect of Strontium on the Bone

The progressive and rapid deviations from the normal in the histology of growing bones due to the feeding of strontium are shown in figures 5 - 9, and are described in the legends accompanying them. Therefore, only a brief general statement concerning the anatomical changes is necessary here.

The most striking feature and the one which appeared most promptly when strontium was introduced into a diet containing little calcium, was a sudden increase in the proliferation of the cartilage cells on the diaphyseal border of the cartilage disk.

This disk in normal bone in which growth is arrested is not more than three or four cells deep; it is about three or four times as

Figure 5

Photomicrograph of a section of a bone of a young rat which was fed strontium for four days before death. Note the increased width of the cartilage band, and especially the tendency to increased width of that portion of the cartilage which is in the vesicular stage, and is prepared for the provisional deposition of calcium salts in the ground substance between the cells. This provisional calcification is still evident.

At the extreme right there is an area consisting of marrow elements and osteoid, which represents the intrusion of these elements into the region between the growing cartilage of the epiphysis and the shaft, which constitutes the metaphysis seen in rickets. There was already an increase in the amount of osteoid around the trabeculae of bone.

Fig. 5

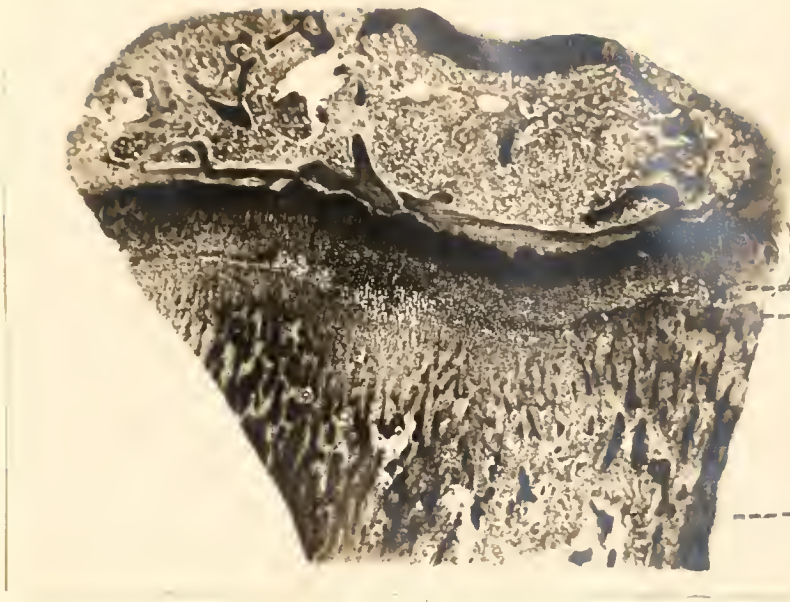
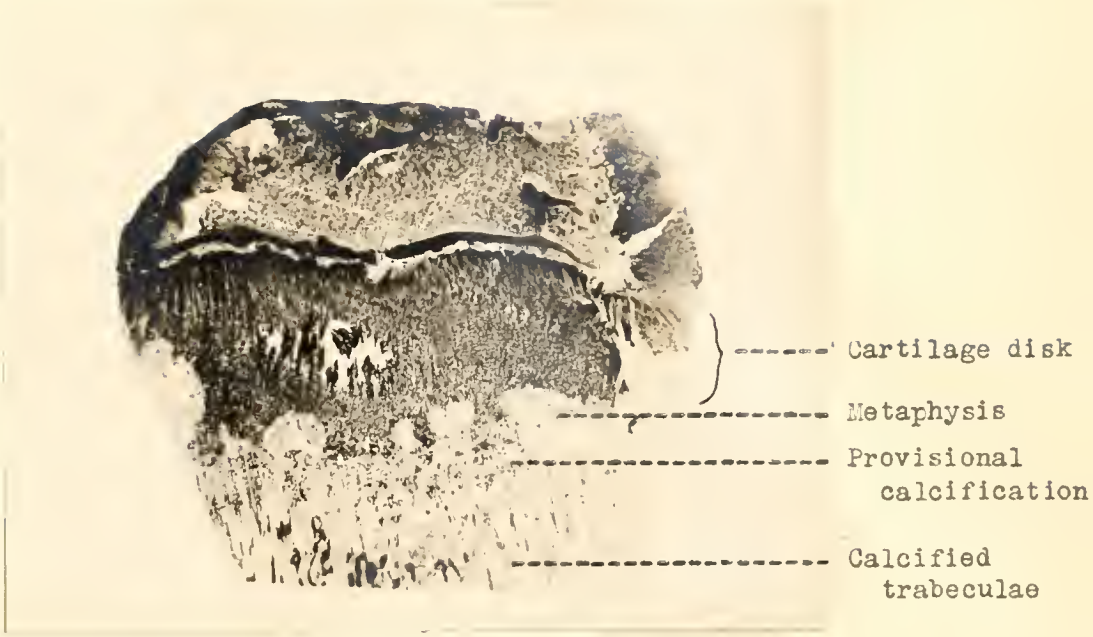

Proliferating car
Metaphysis
Provisional
calcification
Marrow cavity

Figure 6

Photomicrograph of tibia of a rat which had been fed strontium for thirteen days. The cartilage border has more than trebled in width. The line of provisional calcification has completely lost its regularity, and almost disappears at the right of the shaft. Thousands of osteoblasts invade this region. They lie very thick along the edges of the persisting cartilage. Areas of metaphysis are very pronounced in the region between the growing cartilage and the shaft. Calcified bone has entirely disappeared in the right hand side of the shaft, being replaced by osteoid. Small calcified trabeculae surrounded by very wide borders of osteoid still remain in the left. The bone trabeculae have lost to a large degree their orderly arrangement with axes parallel to the bone. Their structure is now more like a web consisting of roundish splash-like calcified areas. In the clear area on the right of the shaft this arrangement becomes definitely sclerotic, that is, there is a mingling of osteoid trabeculae, marrow elements, and blood vessels but with a more or less regular and lattice-like arrangement of these different constituents. The medullary cavity has been invaded by this spongiosa, and the marrow elements are found throughout the shaft.

Fig. 6


Cartilage disk
Metaphysis
Provisional calcification
Calcified trabeculae

wide in one that is growing. Of these cartilage cells about one-half the number are in the so-called vesicular stage, in which they appear swollen and crowded, and present a form somewhat distorted from the globular. The remaining cells are somewhat flattened and are in the resting stage.

In strontium-fed animals the number of cartilage cells in the columns which were in the vesicular stage was greatly increased. Thus after four days of strontium administration the cartilage disk was about three times as wide as the normal, and the region of vesicular cells was twelve to fourteen cells deep. After seven days the region of vesicular cells was eighteen to twenty-two cells deep and the region of resting cartilage was eighteen to twenty-four cells deep. A few days later the cartilage disk was extremely wide, and became very ragged and uneven on the diaphyseal border. The cartilage cells tended to persist, but underwent metaplasia into osteoid. The

Figure 7

Photomicrograph of the bone of a rat which had received strontium for twenty-eight days. The conditions seen in Figure 6 have been greatly extended. The proliferating cartilage disk has widened enormously, and the areas of metaphysis have become very large. Some provisional calcification is seen. The condition seen here is only slightly less severe than that shown by Figure 4. (The effect of thirty-one days of strontium feeding).

Fig. 7

result was that there were tongues and many islands of cartilage cells extending into or surrounded by osteoid, and marrow elements which intruded from the medullary cavity of the shaft and formed the so-called metaphysis seen in rickets and related conditions of bone pathology. The amount of osteoid tissue which was formed was enormous. Calcium deposition was markedly interfered with, but for a time there were irregular deposits in the matrix in which the cartilage cells lay imbedded. This is the type of calcium salt deposition spoken of as provisional calcification.

Our feeding period for strontium covered, in general, thirty-one days. At that time the tongues of cartilage which persisted were often as much as fifteen to twenty times as wide as the normal cartilage disk. The cartilage surrounding the epiphysis was four or five times as thick as the normal. From the beginning of the feeding period the osteoid progressively increased

Figures 8 and 9

Figures 8 and 9 are photomicrographs of mature animals receiving strontium for eleven and nineteen days respectively. They show that strontium affects the mature bone in exactly the same manner but not with the same rapidity that it does the young one. The cartilage disk for the eleven day rat has scarcely more than doubled, while for a similar younger one it had trebled. At nineteen days the proliferating cartilage of the older bone (Figure 9) has about trebled in width. Provisional calcification is quite distinct; spicules of normal bone still remain, and the marrow cavity is almost intact. Several areas of metaphysis are at hand.

Fig. 8

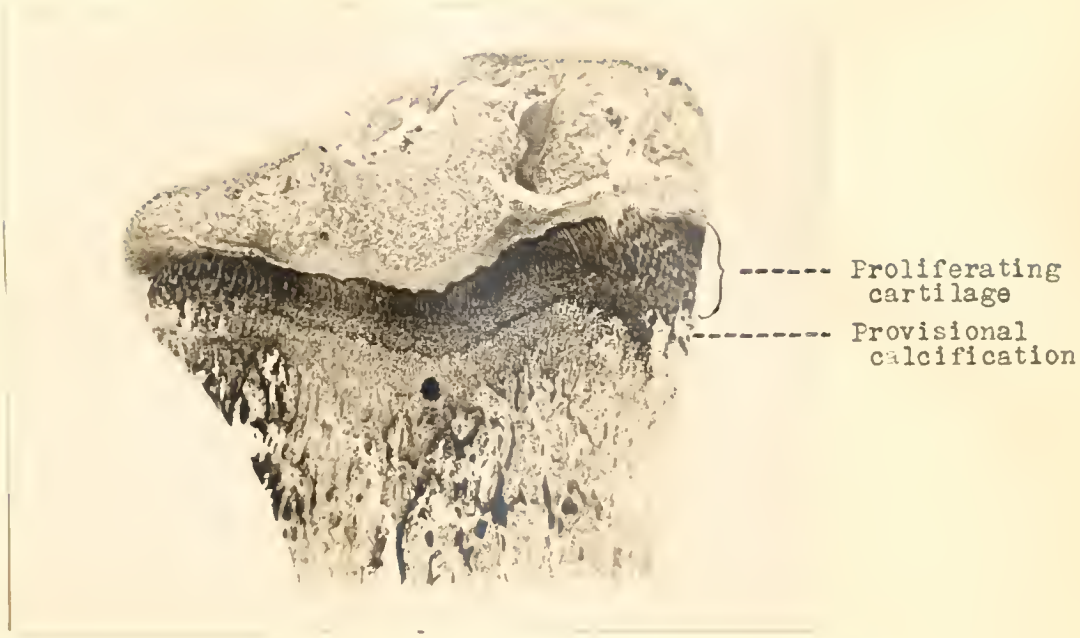
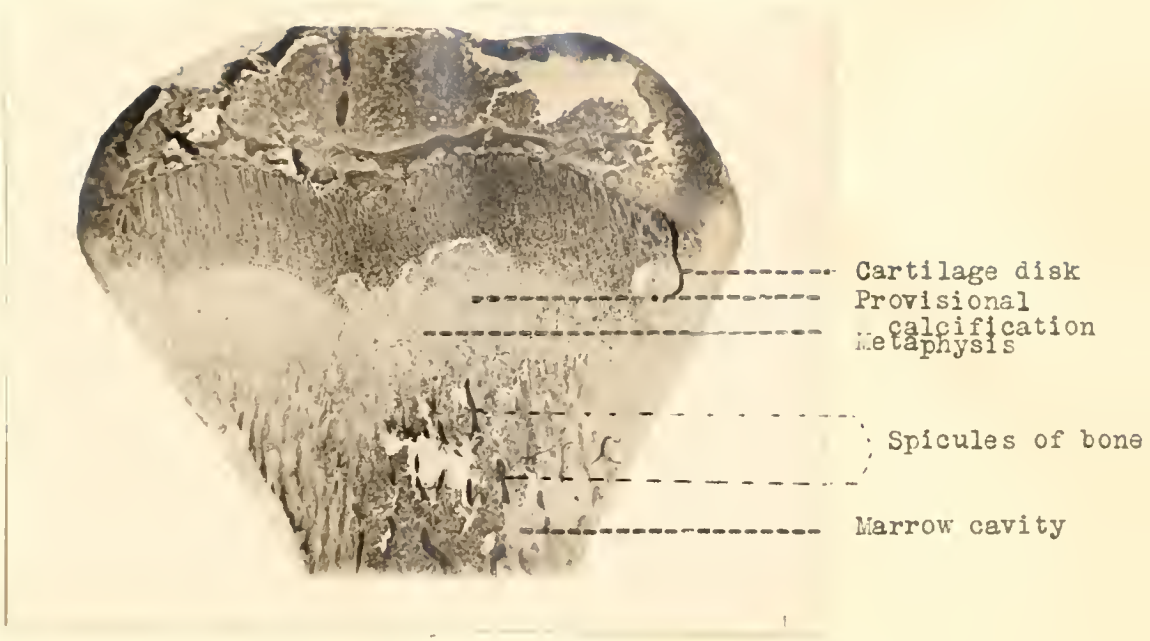


Fig. 9


Cartilage disk
Provisional calcification
Metaphysis
Spicules of bone
Marrow cavity

until by the thirty-first day the trabeculae of bone were practically lacking in the growing region of the bone. The rapidity of the effect of strontium on mature bone is shown in figures 8 and 9. The older bone is not so quickly affected as is the younger one, although the final result is the same. We cannot say that this statement applies to bones that have ceased growing.

Duration of Pathological Changes Produced by Strontium

In order to study the duration of the lesions produced by strontium, a group of young rats which had received the low calcium, strontium-containing ration for thirty-one days were placed on the ration containing an optimal amount of calcium. These rats were then killed at successive intervals of two weeks.

Two weeks after the omission of strontium and the provision of calcium, the enormously wide proliferating cartilage disk seen in

the thirty-first day strontium picture was reduced to a disk very little wider than the normal. A heavy line of provisional calcification crossed the entire width of the shaft in a regular and normal manner. The spongiosa seen in the shaft at the end of thirty-one days of strontium feeding persisted, but there was evidence of resorption and some small deposition of calcium. The cortex was sclerotic.

Four weeks after the cessation of strontium feeding, the medullary cavity was clearly delineated and confined to its normal region. Some few small spicules of bone were being formed in the shaft. The cortex still showed evidences of sclerosis but resorption was taking place very fast. The trabeculae of cartilage in the growing part of the bone were in orderly arrangement and showed evidence of normal bone formation.

Six weeks after the cessation of strontium feeding and the

provision of calcium, the bone was clearly approaching the normal in its structure. The proliferative cartilage border and the line of provisional calcification were normal in all respects. More spicules of bone had been formed. The bone was, however, still somewhat rarified. The only distinct evidence of abnormality lay in the thinness of these trabeculae, and in the cortex which was still somewhat sclerotic.

After twelve weeks the spicules of bone had not quite reached the normal number, but there were more of them than were seen at six weeks. The cortex at that time, rather than being sclerotic, presented a solid shaft of bone. During this interval, therefore, the effects of the administration of strontium had been in a great measure erased.

Our study of the histology of the bones of rats under the different conditions of strontium feeding show that the effect of this element is exceedingly rapid and spectacular. In thirty days it

produces a condition of the bone, which in all its essential characteristics, is rachitic.

When the strontium is withdrawn from the diet and calcium is substituted, there is a rapid resolution of the bone substance, and apparently a pronounced deficit of calcium for a short time, notwithstanding the liberal provision of this element in the food. This appears to be the means adopted by the body for getting rid of the offending strontium. The result is a marked osteoporosis which lasts only for a short time, for as soon as the greater part of the strontium is eliminated, calcification of the bones sets in anew, and after a period of ten weeks from the time of relief from the administration of strontium the bones have been in great measure recalcified. Along with this there is a rapid resorption of osteoid and sclerotic tissue as the bones return toward the normal.

The chemical, physiological, and histological findings are

all in harmony with this explanation of the course adopted by the anatomic elements of the osseous tissues when under the influence of the experimental conditions described in this paper.

SUMMARY OF CONCLUSIONS

1. Strontium is deposited in the bones of an animal which has ingested it. The maximum amount of strontium which the bones will retain is about 7 to 8 per cent. It may enter the body either through the alimentary tract, the placenta, or, in the nursing young, through the milk. Its entrance is not inhibited by the presence of calcium in the diet, but it is taken up by the bones of the adult at a slower rate than by those of the growing young. This is due to the fact that strontium, when it is deposited in the bones, substitutes for calcium.

2. Strontium has a specific effect on the teeth, in that the incisors of the rat lose their color under the influence of the feeding

of this element. The other teeth are also profoundly damaged as the result of its ingestion.

3. When strontium is ingested without a calcium supply there follows a characteristic loss of muscular coordination, and defects of locomotion.

4. Strontium is a powerful stimulant for the cartilage cells in the proliferative zone of the bones, and leads, when the diet is poor in calcium, to their rapid proliferation and metaplasia into osteoid. The metaplasia is, however, decidedly perverted, and many of the cartilage cells persist, but lose their characteristic staining properties.

5. When, after a period of strontium feeding, in which the bones are made to retain about 7 per cent of strontium, this element is omitted from the diet, and calcium is substituted, there is a rapid elimination of both calcium and strontium, leading to

an osteoporotic condition of the bones. As soon as the greater part of the strontium is gotten rid of, a rapid recalcification of the bones takes place and the latter return within a few weeks to an essentially normal condition.

6. When the diet contains both the optimal amount of calcium, and an equimolecular amount of strontium, the calcium tends markedly to inhibit the effects of the strontium.

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